Antibacterial Activity of Ticagrelor in Conventional Antiplatelet Dosages Against Antibiotic-Resistant Gram-Positive Bacteria

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Ticagrelor reversibly inhibits the platelet adenosine diphosphate $P2Y_{12}$ receptor ($P2Y_{12}$).[1] It is approved for prevention of cardiovascular events in patients with atherosclerotic cardiovascular disease and shows evidence of superior clinical performance compared with other $P2Y_{12}$ inhibitors. A post hoc analysis of the Comparison of Ticagrelor (AZD6140) and Clopidogrel in Patients With Acute Coronary Syndrome (PLATO) trial[2] revealed that patients treated with ticagrelor had a lower risk of infection-related death than those treated with clopidogrel bisulfate.[3] More recently, in the Targeting Platelet-Leukocyte Aggregates in Pneumonia With Ticagrelor (XANTHIPPE) study, ticagrelor was associated with improved lung function in patients hospitalized for pneumonia.[4] We therefore questioned whether ticagrelor or its metabolites could possess antimicrobial properties.

Methods

Ticagrelor and its major metabolites (M5 AR-C133913, M7, M8 AR-C124910)[5] were synthetized and tested in time-kill assays against gram-positive methicillin-resistant *Staphylococcus epidermidis*RP62A (MRSE) (ATCC 35984); methicillin-sensitive *Staphylococcus aureus* (MSSA) (ATCC 25904, ATCC 6538); glycopeptide intermediate *S aureus* (GISA) Mu-50 (ATCC 700699); methicillin-resistant *S aureus* (MRSA) (ATCC BAA-1556); *Enterococcus faecalis*(ATCC 29212); vancomycin-resistant *E faecalis* (VRE) (ATCC BAA-2365); and *Streptococcus agalactiae* (ATCC 12386) and against gram-negative *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (PAK laboratory strain). Biofilm formation was assessed in vitro with crystal violet staining and in a mouse model of *S aureus* polyurethane-implant infection using Xen-29 bacteria (Perkin Elmer). Infected disks were implanted in specific pathogen-free BALB/cAnCrl mice (Charles River). The mouse protocol was approved by the ethical committee of Liège University.

Results

Ticagrelor and AR-C124910 had bactericidal activity against all gram-positive strains tested, including drug-resistant strains GISA, MRSE, MRSA, and VRE. The minimal bactericidal concentration was 20 µg/mL against MSSA, GISA, MRSA, and VRE; 30 µg/mL against MRSE; and 40 µg/mL against *E faecalis* and *S agalactiae.* Although a dosage of 5 µg/mL delayed growth of MRSA, ticagrelor was ineffective against gram-negative strains in concentrations up to 80 µg/mL. At minimal bactericidal concentration, ticagrelor was superior to vancomycin (Figure 1A), with rapid killing of late-exponential-phase cultures of MRSA (time to kill 99.9% of the initial inoculum, 2 hours). Bactericidal activity was similar to the bactericidal cyclic lipopeptide

daptomycin, recently introduced against resistant strains of *S aureus* (Figure 1A). A subminimal bactericidal concentration of ticagrelor (10 µg/mL) combined with vancomycin (4 µg/mL) killed approximately 50% of the initial MRSA inoculum, depicting synergistic activity. Ticagrelor also increased the bactericidal activity of rifampicin, ciprofloxacin, and vancomycin in a disk diffusion assay. It displayed bactericidal activity against MRSE and VRE (Figure 1B and C), with superiority over vancomycin for killing MRSE. At 24 hours, its ability to kill MRSE and VRE was similar to daptomycin (Figure 1B and C). Ticagrelor inhibited MRSA, MRSE, and VRE biofilm formation in a dose-dependent manner (Figure 1D-F); biofilm mass was reduced by more than 85% after exposure to 20 µg/mL ticagrelor. Finally, in mice, conventional oral antiplatelet dosages of ticagrelor (3 mg/kg loading dose, then 1.5 mg/kg twice daily) inhibited biofilm growth on *S aureus*–preinfected implants and dissemination of bacteria to surrounding tissues (Figure 2).

Discussion

We describe bactericidal activity of ticagrelor against antibiotic-resistant gram-positive bacteria that pose a threat to human health. Although bactericidal concentrations are not reached systemically in patients receiving typical dosages for treating cardiovascular disease (ticagrelor C_{max}=1.2 µg/mL after one 180-mg loading dose and 0.75 µg/mL at 90 mg twice daily steady state), antibacterial activity at infection sites may still be achieved through local, possibly platelet-driven, drug accumulation. Our findings provide a mechanistic explanation for the reduced infection-related death with ticagrelor seen in the PLATO trial[3] and could also explain improvement in lung function in patients with pneumonia who took ticagrelor in the XANTHIPPE study.[4] These findings warrant further investigations, including design of randomized clinical trials comparing the protective activity of ticagrelor against gram-positive bacterial infection in patients with cardiovascular disease with other antiplatelet drugs. We are unaware of similar findings with other $P2Y_{12}$ inhibitors, and we did not observe in vitro antibacterial activity of the active metabolite of prasugrel in concentrations up to 100 µg/mL. Ticagrelor might prove superior to other $P2Y_{12}$ inhibitors in patients with cardiovascular disease at risk for gram-positive bacterial infections such as infective endocarditis.[6] We did not isolate mutants resistant to ticagrelor, and serial passaging of MSSA or MRSA in the presence of subinhibitory concentrations of ticagrelor did not select for resistant mutants compared with ofloxacin or rifampicin, which is reassuring for long-term antiplatelet indications. There is a main limitation in this study that will be addressed in future research. The in vivo demonstration of antibacterial activity of ticagrelor antiplatelet dosages was obtained in the mouse, which differs from humans in terms of ticagrelor pharmacokinetics. Notwithstanding, our findings also encourage future investigation of potential new ticagrelor-derived antibiotics, devoid of antiplatelet activity, against multiresistant staphylococci or enterococci.